THE EFFECTS OF CHEMICALS ON THE DIVISION RATE OF CELLS WITH ESPECIAL REFERENCE TO POSSIBLE PRE-CANCEROUS CONDITIONS.*

GARY N. CALKINS, FREDERICK D. BULLOCK,
AND
GEORGE L. ROHDENBURG.

(From the Department of Zoology, Columbia University, New York City.)

Three years ago one of us carried out some experiments on regeneration in single cells, using for the purpose the ciliated protozoon *Uronychia transfuga* St. It was found that the power of regeneration varies at different periods of cell life, being lowest immediately after division and greatest immediately prior to division. From these results the conclusion was drawn that the power of regeneration is bound up with the accumulation of some product of metabolism which reaches a condition analogous to saturation just before division and is exhausted by the process of regeneration involved in the reconstruction processes after division. This led to the further hypothesis that division itself might be bound up with the accumulation of some product of metabolism.

As the central problem of cancer research is connected with the exciting cause of cell division we decided to try out the hypothesis by some direct experiments. The obvious chemicals to employ in such a test are the products of nucleo-protein breakdown, including amino acids, nucleins, and their derivatives. In the present paper we wish to present some of the results obtained in these experiments which were conducted on single-celled organisms, and on the mature tissues of peritoneal organs of rats. For many of the pure chemicals used we are indebted to the courtesy of Dr. P. A. Levene of New York, and Dr. Walter Jones of Baltimore.

THE EFFECTS OF CHEMICALS ON THE DIVISION RATE OF ACTINOBOLUS.

One of the free living organisms used in the experiments is an extremely rare ciliated protozoon *Actinobolus radians* Stein. This form has an advantage over other types of protozoa for the present

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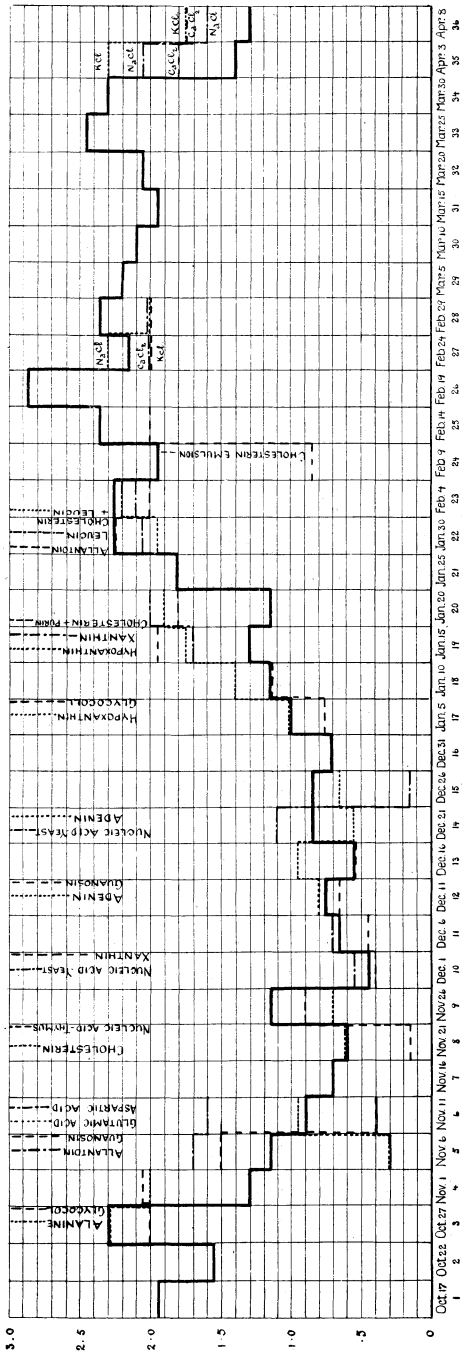
work in that it is not a bacteria-eater but is restricted to animal food, ingesting no other kind of food than the protoplasm of *Halteria grandinella*, another ciliated protozoon.

One of these protozoa was captured and isolated in a few drops of filtered pond water on October 12, 1911. It was fed with Halteria and placed in a moist chamber. In a day or so it had divided several times and the daughter-cells were isolated in watch glasses, supplied with pond water and food as before. This procedure has been repeated daily since that time, the race now being in the 375th generation of cell divisions. Several pure lines were started, four of which have never been tampered with, and these have served as control lines for all of the experimental work. The history of these four lines, as shown by the average division rate, is shown in the accompanying diagram. In this the ordinates represent the average daily rate of division for each individual cell of the four lines; these in turn are averaged for periods of five consecutive days. The abscissae represent the successive five-day periods from October 17 until April 8.

The beginning of the curve on October 17 shows that during the first five-day period the individuals were dividing at the rate of 1.95 divisions per day. This rate is better expressed, for purposes of comparison, by the statement that the organisms were dividing at the rate of 19.5 divisions in 10 days, and this form will be followed in the sequel. In the second period the rate fell to 15.5, but rose again in the third period, when the first experiments were made, to a rate of 23 divisions in 10 days. From this high rate the rate of division declined in successive periods to the lowest rate reached during the course of the experiments, viz., 4.5 during the 10th period. From this low point the division rate slowly increased to a maximum of 28 divisions in the 26th period, in February, and this high rate was again succeeded by a gradually decreasing rate.

The control curve thus shows a characteristic rhythm of growth energy with one period of depression during November and December, a result which has been obtained with practically all similar cultures of protozoa, and a phenomenon which has been recognized in connection with various kinds of rapidly dividing cells, even in


3.0
2.5
2.0
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Oct. 17 Oct. 22 Oct. 27 Nov. 1 Nov. 6 Nov. 11 Nov. 16 Nov. 21 Nov. 24 Dec. 1 Dec. 6 Dec. 11 Dec. 16 Dec. 21 Dec. 26 Dec. 31 Jan. 5 Jan. 10 Jan. 15 Jan. 20 Jan. 25 Jan. 30 Feb. 4 Feb. 9 Feb. 14 Feb. 19 Feb. 24 Feb. 27 Mar. 5 Mar. 10 Mar. 15 Mar. 20 Mar. 25 Mar. 30 Apr. 3 Apr. 8
ALANINE
GLYCOCOLL
ALLANTOIN
GUANOSIN
GLUTAMIC ACID
ASPARTIC ACID
CHOLESTERIN
NUCLEIC ACID HAMMERSTEN
NUCLEIC ACID TOTHILS
NUCLEIC ACID YEAST
XANTHIN
ADENIN
GUANOSIN
NUCLEIC ACID YEAST
ADENIN
HYPOXANTHIN
GLYCOCOLL
HYPOXANTHIN
XANTHIN
CHOLESTERIN + PUMIN
ALLANTOIN
LECITIN
CHOLESTERIN + LECITIN
CHOLESTERIN EMULSION
NaCl
KCl
CaCl2
NaCl
KCl
CaCl2
NaCl
KCl
CaCl2
NaCl

cancer cells, according to the observations of Bashford, Hertwig and Poll, and others.

In the experiments, the individuals to be treated with chemicals were in every case daughter-cells of individuals of the control lines of the same date. The lethal dose of the chemical to be tested was first ascertained by experiment, and was then diluted to such an extent that neither Actinobolus nor its food Halteria was killed by 24 hours' exposure. The same quantity of pond water and food was used and every other condition was the same as with the control lines, the only difference being the addition to the medium of the chemical experimented with. Four lines of cells were used in every test and four different strengths of the chemical. The tests extended in every case over a period of 10 days, thus giving two five-day periods for comparison with the control. For this comparison we have used only the average of the four experimental lines; in many instances more marked differences between the experimental and the control series would have been obtained had we used only that strength of chemical which gave the most pronounced reaction.

For the best analysis of the results we will compare the reactions of the chemicals at different periods of vitality of the protoplasm in the control lines. Four such periods are evident from the curve: first, a period of decreasing vitality as indicated by a rapidly descending division rate from October until the end of November (periods 1–9 inclusive); second, a period of low vitality during the month of December (periods 10–16 inclusive); third, a period of increasing vitality from the first of January to the end of February (periods 17–26 inclusive); and fourth, a period of decreasing vitality from the end of February to the middle of April (periods 27–36 inclusive).

Effects of chemicals during the first period of decreasing vitality.— During this period eight chemicals were tested: alanin, glycocoll, allantoin, guanosin, glutamic acid, aspartic acid, cholesterin, and nucleic acid from the thymus. Alanin and glycocoll were used with a maximum strength of $\frac{N}{10}$, one drop to five drops of food medium. During the two successive five-day periods under treatment the

control lines dropped from a rate of 23 divisions in 10 days to a rate of 13 divisions. The sister-cells treated with alanin divided the same number of times as the control during the first period and the rate then fell to 20 divisions, or seven better than the control. Those treated with glycocoll maintained a steady rate of about 20 divisions in 10 days, throughout the experiment. These monobasic amino acids, therefore, appear to have had the power of sustaining vitality when the division rate of the unstimulated protoplasm was rapidly falling. That the action was not due to these complex substances acting as food was proved by the simple experiment of omitting the Halteria from the food medium and using only pond water with the chemical. In the specimens thus treated the division rate was the same as that of the control for the first 24 hours but after this not one of the individuals divided again and all died from starvation within 72 hours.

The purins guanosin and allantoin were used in the form of saturated solutions. This, for allantoin, was diluted 1:10 and used in the maximum strength of one drop to five. Guanosin, diluted 1:5, was used in the maximum strength of one to four. Specimens treated with allantoin divided at the rate of 17 and 16 divisions in 10 days, while those treated with guanosin divided at the rates of 15 and 4 for the two periods of five days each. The control lines divided at the rates of 11.5 and 9. It is significant that the greater effect of stimulus is shown by allantoin, a later product of nucleo-protein breakdown.

A well marked depressing effect was produced by the dibasic monamino acids, glutamic, and aspartic, the rates for which were 3 and 9 for glutamic, and 3 and 4 for aspartic, as against 11 and 9 for the control. These acids in saturated solutions were diluted 1:20, 1:40, 1:80, and 1:160 and used one drop to five.

Cholesterin and nucleic acid from the thymus, used in saturated solutions diluted 1:30, one drop to five, had but a slight effect on the division rate at this time.

Effects of chemicals during the period of low vitality.—During the periods from 10 to 16, the division rate of the control averaged a little less than 7 divisions in 10 days, the lowest rate for a five-day period being 4.5, the highest 8.5 divisions in 10 days. The

chemicals used were nucleic acid from yeast (periods 10 and 11), xanthin (periods 10 and 11), adenin (periods 12 and 13), and guanosin (periods 12 and 13). Saturated solutions were used in all cases. During these periods the nucleic acid and xanthin had little appreciable effect. Adenin had a curious effect, giving a slight stimulus in periods 12 and 13, and a depression in periods 14 and 15. Guanosin, also, had but slight effect, if any, at this period. With these chemicals at this time, therefore, the variations from the control are too minute and too indefinite to warrant conclusions.

Effects of chemicals during a period of increasing vitality.— During this third period of the history of Actinobolus the division rate rose from a low rate of 7 divisions in 10 days in the first period in January (period 16) to a maximum rate of 28 divisions in the fourth period in February. The increase was steady during this time and has as steadily declined since then.

The most interesting results at this time were obtained with the chemicals hypoxanthin and xanthin, both in saturated solutions diluted 1:100 and 1:80 respectively. Examining the action of hypoxanthin first the control curve shows that both control and chemically treated individuals were dividing at the rate of 10 times in 10 days. In the second period of treatment (period 18) the relation was 11.5 control and 14 hypoxanthin. In the third period (19) the relation changed to 13 and 17.5 respectively, while in the fourth period the control fell to 11.5 and the hypoxanthin-treated cells rose to 19.

The individuals treated with xanthin showed a similar stimulation, the control dividing at the rate of 13 divisions in 10 days (period 19) during the first period while the treated cells rose to 17. The difference was still more manifest in the second five-day period (20) when the rate for the control fell to 11.5 and that of the xanthin-treated individuals rose to 20. When this result is contrasted with that obtained with xanthin during the period of depression in December (periods 10 and 11) when a still greater depressing effect was produced by the chemical, the comparison is interesting and significant and indicates that the factor of vitality must be taken into consideration in all experiments on the effects of chemicals.

A similar high division rate was obtained with cholesterin mixed with xanthin and hypoxanthin. The potency of the mixture being due, presumably, to the action of the purins, for cholesterin when used alone had a depressing effect on the division rate (see periods 8, 9, and 24, 25).

Allantoin, which had been found to have a stimulating effect when the protoplasmic vigor was declining, was again tried when the rate was high (22.5 divisions). There was no effect during the first five days, the division rate remaining the same as the control, but in the second five-day period the individuals treated with allantoin fell to a rate of 20 while the control remained the same (periods 22 and 23). Leucin, tested at the same time, brought the rate down to about 20 divisions for the 10 days.

Summarizing in a word the results on free living cells, there seems to be little doubt that, under certain conditions of protoplasmic activity, some chemical products obtained by the hydrolysis of nucleo-proteins have a definite stimulating effect upon the division rate of free living cells. At the present time we have no hypothesis to offer as to the interpretation of the chemical reactions involved nor do we wish to make any attempt to harmonize our initial working hypothesis with the results obtained. It is certainly significant, however, that the later products of the hydrolysis of nucleins have the greatest stimulating effect upon the division rate.

THE EFFECTS OF CHEMICALS ON TISSUE CELLS.

There are many difficulties to overcome in getting a successful local application of chemicals on tissues in the living body. The agar-agar method, which we finally adopted, is an adaptation of one described by Leo Loeb in 1905. This method has the advantages of permitting a uniform mixture of the chemical; of allowing the permeation by new cells in their growth, thus enabling them to come in direct contact with the chemical; and of firmly holding insoluble or slightly soluble chemicals, thus preventing or retarding their removal by phagocytes and prolonging their chemical action.

The chemical or chemicals to be tested were thoroughly mixed in a 2 per cent agar jelly. Of this mixture 2 c.c. were injected

while the agar was still liquid (usually at a temperature of about 44° C.) in the subcutaneous regions of the breast or in the peritoneal cavity of normal rats. In all experiments, except when otherwise specified, 0.5 gm. of the chemical was mixed in 15 c.c. of agar.

For each experiment sometimes four, sometimes five adult animals were used and killed at different intervals; the injections were invariably made under ether anaesthesia and the animals were always killed while under ether. Complete autopsies were made in every instance and all tissues showing changes in the gross were carefully fixed in sublimate acetic or some other equally good cytological fixing fluid.

The chemicals were used either in combination with cholesterin or alone. Those used with cholesterin are listed on p. 430; those used alone included the following: cholesterin, glycerin, skatol, methylglycin, tyrosin, amido-acetic acid, creatin, cystin, leucin, sodium amido formate, asparagin, aspartic acid, ammonium carbamate, alloxanthin, hippuric acid, lecithin, guanin, guanin hydrochloride, xanthin, uric acid, and urea.

As the results after subcutaneous and intraperitoneal injection involve somewhat different reactions they may be more conveniently described under the headings "Subcutaneous Reactions," and "Intraperitoneal Reactions."

Subcutaneous reactions.—In this series of experiments all of the single chemicals enumerated above were used with agar. The dosage was the same in the case of all chemicals except guanin hydrochloride in which it was reduced to 0.25 gm. in 15 c.c. of agar.

In all cases the gross pathological changes are the same. After 10 days' exposure the injected material is surrounded by a firm, dense capsule varying in thickness from 0.5 to 5.0 mm. Ingrowing strands of connective tissue cells invade the agar in all directions. With longer exposure these strands become more dense and more firmly knit until by the 30th day the mass of agar in some cases is quite replaced by newly formed tissue.

A typical illustration of the microscopic picture is given by the reaction following injection of agar asparagin. At the end of 10 days the agar is largely replaced by good sized cells of rounded or

polyhedral form and with indefinite margins. These may contain one rounded or ovoid nucleus or there may be many such arranged about the periphery. The cytoplasm takes a deep acid stain with eosin and may inclose fragments of agar. Mitoses are frequently seen. These giant cells, possibly derived from polyblasts, are numerous and closely packed together in some areas, being separated only by a thin stroma of connective tissue cells. In some areas, particularly at the periphery of the agar, such giant cells may fuse, forming syncytia of various sizes and of fantastic shapes, and all with indefinite cell walls. The relatively few nuclei of such masses are arranged about the periphery. The development of these syncytia has been completely worked out by Mallory, Wolbach, and others, and need not be considered further.

The deeper portions of the agar are invaded by leukocytes, polyblasts, and other wandering cells, which become reduced in number, however, with longer exposure. In short, the result may be regarded as a granulation tissue reaction with capillary, fibroblast, and giant cell formation. The connective tissue is finally condensed into a firm capsule while the agar is more and more organized by the ingrowth of cells, with syncytia and giant cells (which sometimes show hyalin and fatty degeneration) distributed here and there.

Similar pictures are produced by guanin, guanin hydrochloride, alloxanthin, tyrosin, creatin, sodium amido formate, leucin, amidoacetic acid, aspartic acid, methylglycin, uric acid, hippuric acid, and urea. Ammonium carbamate causes necrosis of tissue as a result of which the early changes were not observed. With skatol and cystin the early reaction persists throughout. Lecithin produces a milder reaction, the late stages described above not appearing. Cholesterin causes considerable necrosis and degeneration of the tissues, the early changes resembling those described, but scattered through the newly formed tissue are cholesterin crystal spaces around which cholesterin crystal giant cells have formed.

Intraperitoneal reactions.—In these experiments the following chemicals were used singly and in the same strength as with the subcutaneous injections: ammonium carbamate, asparagin, cystin, hippuric acid, uric acid, urea, alloxanthin, and xanthin. Com-

binations of single chemicals with cholesterin were also used in the proportion of 0.25 gm. chemical, 0.25 gm. cholesterin thoroughly mixed in 15 c.c. of agar. The chemicals thus used were: nucleic acid, nuclein, alloxanthin, xanthin, uric acid, urea, amido-acetic acid, ammonium carbamate, aspartic acid, cystin, creatin, asparagin, tyrosin, leucin, and hippuric acid.

Again the gross pathological changes are practically the same in all cases. The injected material forms masses of different sizes and shapes scattered throughout the peritoneal cavity. These are abundant on the upper surface and between the lobes of the liver; on the parietal surface of the spleen; around and between the irregularities of the pancreas, and on different portions of the stomach, intestines, and omentum. The deposits never extend beyond the peritoneal cavity. In animals killed shortly after injection, the agar is loosely attached to the various organs like a mantle; later it becomes firmly attached to the organs and is invaded and enmeshed by newly formed tissues.

The microscopical pictures resulting from the reactions differ according to the tissues involved. For purely descriptive purposes we will describe them under the headings "Peritoneal Reaction," and "Epithelial Reaction."

a) *The peritoneal reaction.*—The reactions produced by different chemicals differ in degree rather than in kind. In some cases giant cell and connective tissue formations are well marked; in other cases this particular reaction is slight (Pl. 2, Fig. 1). In general it may be stated that the peritoneal response is similar to that of the subcutaneous tissues, the essential difference being the unusual proliferation of mesothelial cells in the former, of connective tissue cells in the latter (Pl. 2, Fig. 2).

The mesothelial cells are arranged in layers around the fragments of agar, or invade the agar mass, forming a mesothelial network (Pl. 2, Fig. 3). These cells usually have an indefinite outline and are spherical, discoid, or columnar in form. Large and small syncytia, often of fantastic shape, are likewise formed within the agar mass. These also have an indefinite outline; their protoplasm stains deeply with eosin, and contains relatively few nuclei. Usually such giant cells inclose a particle of agar (cf. Pl. 2, Figs. 1

and 2). Sometimes there is a connective tissue capsule about several foci of reaction thus forming a tubercle-like mass.

In animals killed after 30 days there remains a more or less dense and fibrous structure containing various cellular elements and fragments of agar surrounded by giant cells, many of which are undergoing fatty and hyalin degeneration.

b) *The epithelial reaction.*—The epithelial reaction has to do with the response of connective tissue and functional cells of the peritoneal organs. Partly through the ingrowth of peritoneal connective tissue and partly by proliferation of the stroma of the glands, specialized secreting cells are cut off as islands or long strands from the bulk of the gland. Such isolated cells are usually normal but they may appear in various stages of degeneration. This reaction is quite characteristic of all of the intraperitoneal work, occurring not only when chemicals are present, but also with plain agar controls.

With certain chemicals, used in combination with cholesterin, notably with leucin, ammonium carbamate, uric acid, and urea, the stimulus to cell division is carried not only to the connective tissue elements of the organ but to the secreting cells themselves, thus indicating a far-reaching influence on cellular metabolism (Pl. 2, Fig. 4). This remarkable effect is most striking in the case of the supposedly fixed cells of the liver and pancreas. Innumerable mitoses of the epithelial cells are found in and around areas of granular degeneration involved in the connective tissue reaction. The majority of the mitotic figures are perfectly normal, although here and there degenerating forms may be found. This proliferation probably indicates an attempt on the part of the organ to regenerate. In the liver new bile ducts are formed, from the cells of which or from old gland cells, new liver cells are derived. Such newly formed cells may be found, surrounded by connective tissue, at a considerable distance from the bulk of the organ. In the pancreas marked changes in the histological picture result (Pl. 3, Figs. 5 and 6). In many cases the gland cells, cut off by the invading connective tissue, undergo what may be termed degenerative metaplasia, and become smaller, and flattened or cuboidal, completely losing their characteristic appearance (Pl. 4, Figs.

7 and 8). In other cases the individual cells are less affected, but the acini are irregular in shape and size (Pl. 4, Fig. 8). In such cells mitotic figures are abundant, two or even three in one field of the immersion lens being not uncommon (Pl. 4, Fig. 9). In both liver and pancreas these specific reactions occur mainly in the region of the agar deposit and only rarely in distant parts of the organ.

In addition to the general reaction described above, some of the chemicals produce changes different from others or more marked in extent. With urea there is a clearly defined local degeneration of liver cells together with numerous mitoses, chiefly in the vicinity of the injected mass but evident also in distant parts of the organ, Pancreas cells on the other hand are not thus affected. With leucin, in all of the organs examined, there are in some cases peculiar and highly characteristic reactions analogous to the changes during acute yellow atrophy in the human. There is a widespread degeneration of all the visceral organs, the parathyroids alone excepted; the liver cells are undergoing more or less fatty degeneration, and in the organ as a whole there are innumerable areas of focal necrosis, surrounded by actively proliferating liver cells and newly forming bile ducts. With ammonium carbamate and uric acid the changes are sufficiently described in the general account given in the preceding paragraph.

ANALYSIS OF THE TISSUE EXPERIMENTS.

It is a matter of common belief that mitotic figures in glands like the pancreas and liver are extremely rare. Some authorities, indeed, question the occurrence of mitosis in fully developed normal cells of either organ. A number of questions now arise which demand consideration.

Are there analogous reactions in normal rats?

After finding so many mitotic figures in the experimental animals we began to doubt the traditional belief as to their scarcity in liver and pancreas. To convince ourselves, 10 supposedly normal rats were killed and sections from different regions of the pancreas and liver were made and carefully studied. Of these 10 rats six had parasitic cysts (cestode) of the liver which in every

case showed some degree of degeneration; and four showed a parasitic infection of the pancreas, the exact parasite not being identified. Mitotic figures in the pancreas cells were not found in animals free from parasites, but were occasionally found in the four infected animals, a search of from 15 to 20 minutes being necessary to find one. Mitotic figures in liver cells were somewhat more common, being occasionally present in all of the animals but more numerous in the infected ones.

The liver reaction to parasites deserves a little further consideration. Among the hundreds of rats autopsied 30 per cent were found to be infected, as shown by the presence of liver cysts. The parasite lies in a mass of viscous mucous-like fluid inclosed by a relatively thick connective tissue capsule containing few blood vessels and many plasma cells, some round cells, and leukocytes (Pl. 5, Fig. 10). In the walls of the capsule there are numerous newly formed bile ducts and strands of new liver cells from one to several layers thick. Some of these liver cells are in active mitosis.

From this examination of supposedly normal rats we may conclude, therefore, that mitosis in the pancreas occasionally occurs as a mild regenerative response to injury from parasites. Mitosis is more common in liver cells, presumably as a result of injury by parasites. In general, however, the number of mitotic figures does not compare with that found in these organs in the experimental animals.

Does agar without chemicals induce the same changes?

To determine whether the well marked responses are to be explained as exaggerated foreign body reactions, seven normal rats were given intraperitoneal injections of 2 c.c. of 2 per cent agar, the animals being killed at periods corresponding to those of the chemical series. Parasitic cysts in the liver were found in three of the animals. The gross changes were found to be the same as those already described. The peritoneal reaction also was similar, but the number of syncytial cells was much smaller (Pl. 5, Fig. 11). The characteristic delamination of liver cells was present in several instances but was not found with pancreas cells. Mitotic figures were comparatively rare, none being found in two livers and one pancreas, and only occasionally in the others. In no case

was there evidence of the so-called regenerative process found in the chemically treated animals. The picture, in short, shows no changes apart from the peritoneal reaction, different from our supposedly normal non-injected animals.

Do foreign bodies in the agar act as mechanical stimuli?

If the reactions set up are due to mechanical stimulation, any foreign body with the same physical characters as the chemicals employed should produce a similar result. To test this possibility, pure carbon and powdered silicon were each injected into normal rats, four animals receiving 2 c.c. each of agar carbon in the proportion of 0.25 gm. carbon to 15 c.c. agar, and four receiving an equal dose of powdered silicon. The gross changes were the same as before and the histological picture was practically the same as that produced by plain agar, giant cells, however, being more abundant. The results of the experiment gave no evidence to indicate that a purely mechanical stimulus is responsible for the deeper reactions in the chemically treated animals.

Do artificial products of nucleo-proteins act as stimuli?

It is a significant fact that the most effective chemicals employed to stimulate cell division, both in free living Actinobolus cells and in tissue cells are the later products of nucleo-protein hydrolysis, many of these being purin derivatives (xanthin, hypoxanthin, allantoin, uric acid, ammonium carbamate, and urea). Some of these are readily soluble and it is a question how long they actually act on the tissues before dissolving out of the agar.[1] They all produce cell degeneration and cell death in varying degrees.

The question now arises, *Do the products of cell autolysis give similar results?*

Another series of experiments was carried out in the hope of getting some answer to this question. In three normal animals the splenic end of the pancreas was tightly ligatured and left *in situ*. These we will designate Group A. In three other animals, forming Group B, the end of the pancreas was similarly tied and cut off distally to the ligature. In three other animals, comprising Group C, burnt pancreas tissue was mixed with agar and injected into the

[1] Control experiments with highly soluble dahlia were carried out to determine how long the agar might hold the chemical. Agar deeply colored with dahlia was injected into rats. One died at the end of the fourth day. The agar removed from the peritoneal cavity had lost considerable color, especially around the margins, but the color was by no means all gone, showing that some of the dahlia remained.

peritoneum. One animal of each group was killed on the third day; another was killed (or had died) on the sixth or seventh day, and the third was killed on the 10th day. The gross changes at autopsy do not concern us here. The reaction in rats injected with burnt pancreas and agar (Group C) was in all respects similar to that produced by plain agar alone. In Group B, with pancreas tied off and excised, there was a narrow zone of complete necrosis distal to the ligature and a zone of pancreas cells of about equal width in partial necrosis proximal to the ligature (Pl. 6, Fig. 12). In the zone of pancreas cells adjacent to the latter the following changes were observed: (1) a marked proliferation of the mesothelial elements of the pancreas and of the interlobular and interacinus connective tissue cells; (2) an immigration of leukocytes, plasma cells, and a few mast cells; (3) a shrinkage of the acini and partial degeneration of their cells; (4) loss of the compact organ structure with distinctly separated, irregularly shaped, or distorted acini lying in a reticulated tissue; (5) the presence of double nucleated cells, and cell inclusions bearing a striking resemblance to the cell inclusions found in human carcinoma (Pl. 6, Fig. 14). These cell inclusions were very abundant, as many as six being seen in one section of an acinus. Mitosis in this area occurs, but was not frequently found (Pl. 6, Fig. 13).

In the area adjacent to the last, minor evidences of degeneration were found, especially in connection with the cell membrane, zymogen granules, and chromatin, some of the nuclei, even, taking an acid stain. Here also, there was distortion of the acini, but there was an abundance of mitotic figures.

In Group A, the reaction of the pancreas cells was identical in nature with that of Group B, but the changes were much more extensive and more decisive (Pl. 6, Fig. 15, and Pl. 7, Figs. 16 and 17).

In these experiments, therefore, we have evidence that the presence of cells undergoing autolysis produces the same type of epithelial reaction as that produced by pure chemical products of nucleo-protein hydrolysis. The failure to get the same reaction with burnt pancreas indicates that it is induced by active chemical substances from degenerating protoplasm and not by products of similar protoplasm killed by heat.

GENERAL DISCUSSION.

There has always been a tendency to associate cancer with the biological phenomena of regeneration. This is particularly well brought out in the *Fourth Scientific Report* of the Imperial Cancer Research Fund, in which it is argued that reparative processes following injury and under conditions of chronic irritation may become habitual, resulting in tumor growth. The contributors to this report emphasize the difference between the genesis of cancer and its continued growth. It is not improbable, however, that the factors at bottom are the same.

Our work on tissues has been done with the idea of getting light on the factors having to do with the genesis of cancer. The fundamental cancer problem, like that of regeneration, is expressed by the question: What induces the unusual division of apparently normal cells? The results obtained thus far demonstrate that we can induce mitosis by artificial means in tissues as stable as pancreas and liver, while a remarkable responsive development of mesothelium and connective tissue shows that the reactions involved are not limited to one type of cells, and that they follow the lines of normal regenerative processes.

We are accustomed to look upon unusual mitoses or regenerative processes as responses to stimuli. We know cases where metabolic products of parasites serve as stimuli to such development; for example, crown galls and vegetable galls in general. Such products are toxins belonging probably to the group of nucleo-proteins.

By using products of similar nature in the pure state and free from bacterial contamination, we have produced innumerable mitoses, and other marked changes in the histological picture of tissues ordinarily regarded as fixed, viz., pancreas and liver. We have done in the body what many investigators are successfully doing with epithelial and connective tissue cells on artificial media outside of the body.

We have not produced a tumor—we should like to say that we have not yet produced a tumor—but we have produced, by known means, characteristic changes in normal tissues which might well

represent, and which may yet turn out to be, precancerous conditions.

Every student of cancer has his working hypothesis and there are many theories under which cancer work is progressing throughout the world. They all fail, however, to account for the essential factors in the problem, viz., the origin of the stimulus to division of the latent cell, and the sources of stimuli to the continued proliferation of cells during continued growth, metastasis formation, and transplantation. The advocates of the parasite hypothesis exhaust their energies in a vain search for the parasite. None has been found, while, on the other hand, many intracellular parasites are known, especially the gregarines and coccidia, which have no stimulating effect on the host cells. Advocates of the Cohnheim theory must still explain the sudden stimulus to division of cells of the questionable "embryonic rests," and their explanation would then apply with equal force to the latent normal tissue cells; the same is true of Ribbert's theory. Those who maintain that cancer is due to a change in the biological properties of cells are undoubtedly correct, but here, again, is the same unanswered problem: What induces the change? Theories of fertilization or of rejuvenation of epithelial cells have come and gone, unsupported by fact, and with far-fetched maturation analogies. Those who hold that cancer is due to changed mass relations of nucleus and cytoplasm are working in biological darkness and are unable to see either beginning or end of the cancer problem. Those who gravely state that cancer is due to cells which have lost the habit of function and acquired the habit of growth, although biologically justified, are hopelessly off the firing line, and come dangerously near those pessimists who insist that the cancer problem will be cleared only when the problem of life is solved.

Our experiments on protozoa and on mammalian tissues have been carried out under the stimulus of a more general, more neglected, and, as we believe, a more probable hypothesis than any of the above; this may be outlined somewhat as follows: as a result of abnormal, local, metabolic conditions, brought about by injury, by chronic irritation, by parasites, or by other causes, products of

autolysis are formed which stimulate the division energy of latent cells. This is ordinarily held in check by the regulatory processes of the organism, but with continued irritation the division energy outruns the regulation of the organism and a tumor results. The development of cancer, with the inevitable train of degenerating products, overrunning the regulatory control of the organism, is continued through the activity of the cumulative products of cell autolysis, the degeneration of cancer cells thus producing the stimulating agents for further and more widespread development.

On this theory we find it possible to interpret all kinds of tumors. The obvious weak point is the transition from a reparative process subject to the control of the organism, to a malignant process uncontrolled and unregulated.

EXPLANATION OF PLATES.

PLATE 2.

FIG. 1.—General peritoneal reaction following the injection of agar cholesterin urea. Various stages in the formation of syncytial cells (2).

FIG. 2.—General peritoneal reaction following injection of agar leucin (15 days). *O*, capsule of connective tissue around unorganized agar mass (*P*), *Q*, syncytial cells.

FIG. 3.—Peritoneal reaction following injection of agar cholesterin leucin (15 days). *E*, degenerating liver cells; *D*, proliferating liver cells in suspensory ligament.

FIG. 4.—Epithelial reaction following injection of agar cholesterin leucin (10 days). *A*, liver cell in mitosis.

PLATE 3.

FIG. 5.—Epithelial reaction of pancreas following agar cholesterin urea (15 days). *U*, normal pancreas acini; *V*, isolated acini undergoing degenerative metaplasia; *W*, remains of agar.

FIG. 6.—Peritoneal reaction involving displaced pancreas tissue following injection of agar cholesterin urea (20 days). *A*, island of pancreas tissue showing invasion of connective tissue (*B*); *C*, isolated acini.

PLATE 4.

FIG. 7.—Epithelial reaction following injection of agar cholesterin urea (15 days). *R*, area of degenerative metaplasia; *S*, agar masses; *T*, area of normal pancreas.

FIG. 8.—Epithelial reaction following agar cholesterin urea (10 days). Portion of pancreas showing change from acinous to duct type, with three mitotic figures (2).

FIG. 9.—Epithelial reaction following injection with agar cholesterin uric acid (15 days). Pancreas acinus with three mitotic figures (1).

PLATE 2.

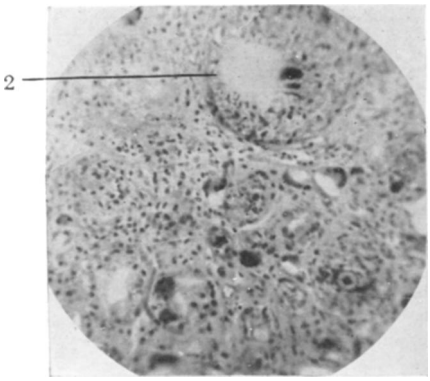
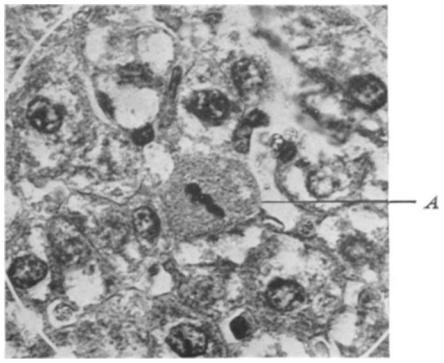


FIG. 1.



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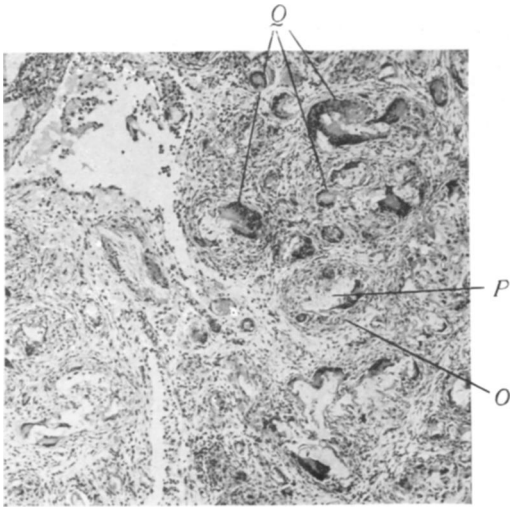

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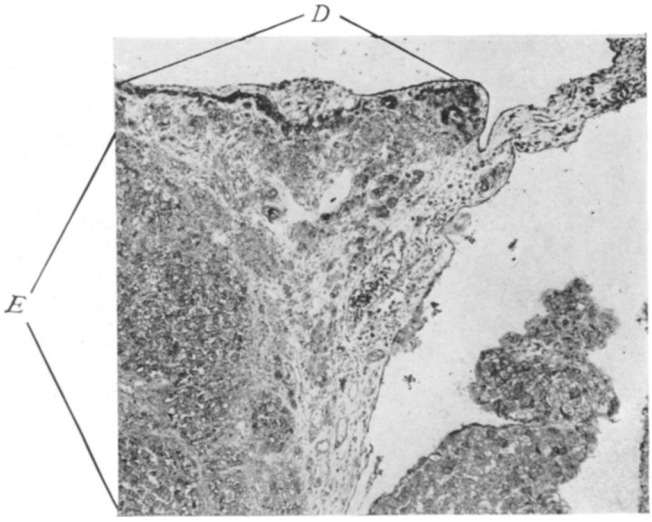
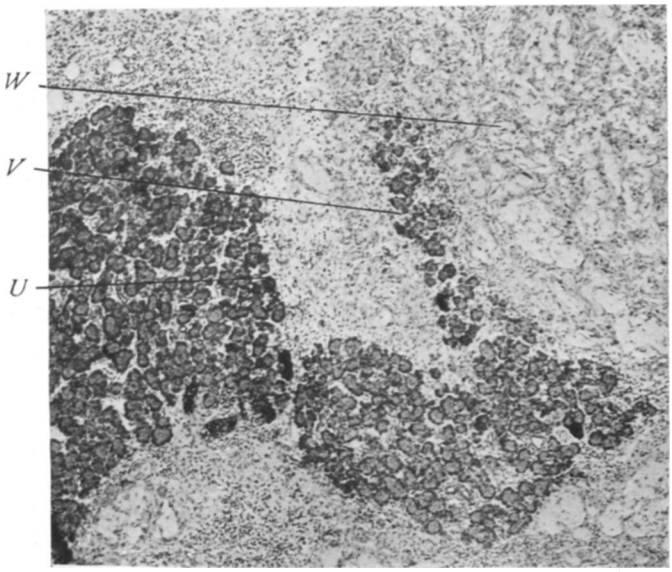


FIG. 3.

PLATE 3.



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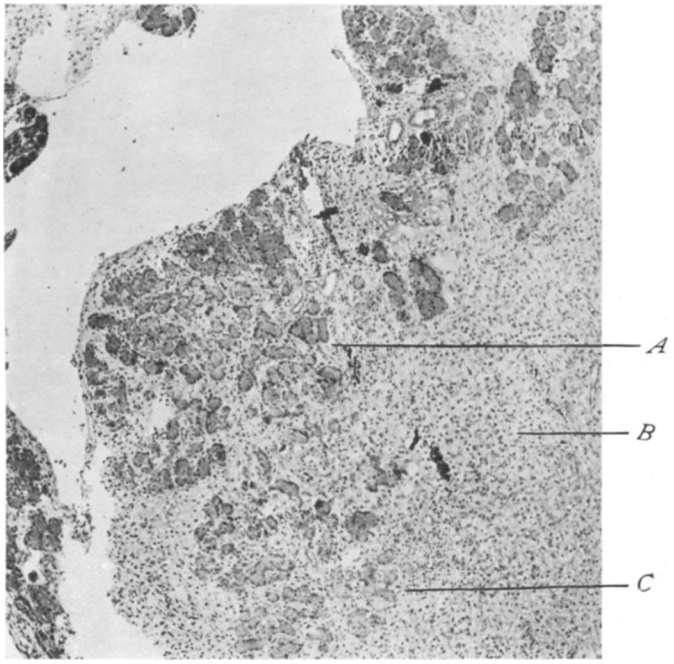
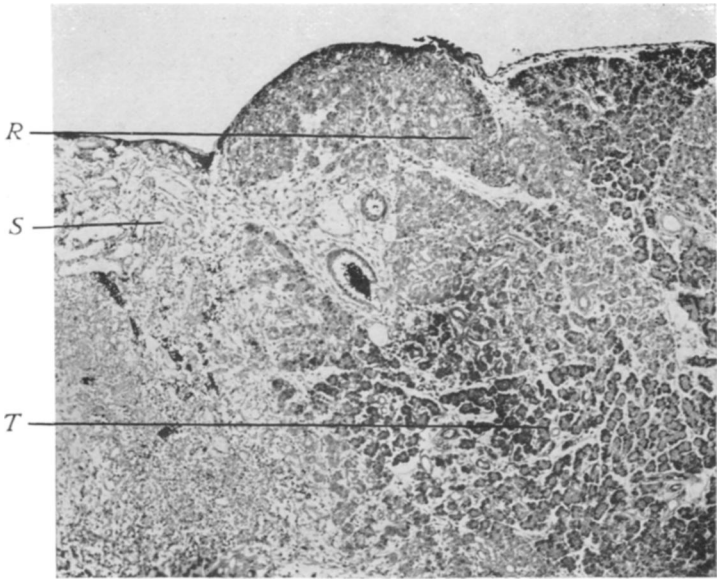


FIG. 6.

PLATE 4.



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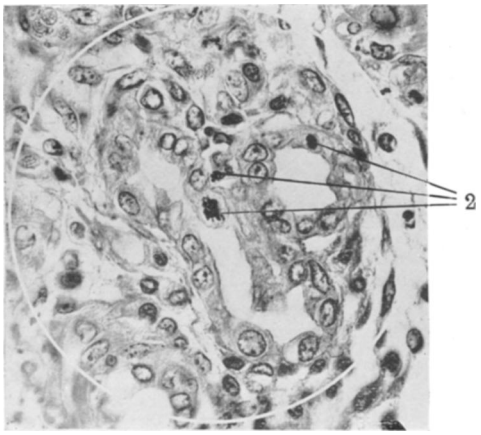

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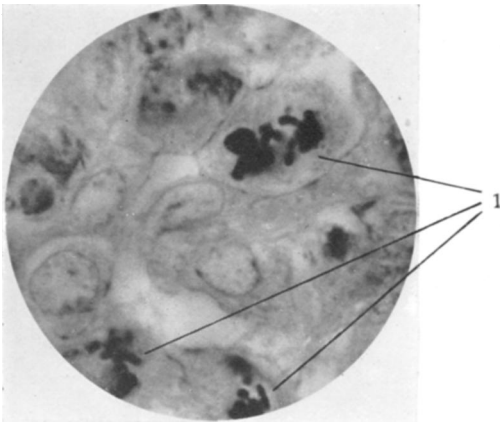
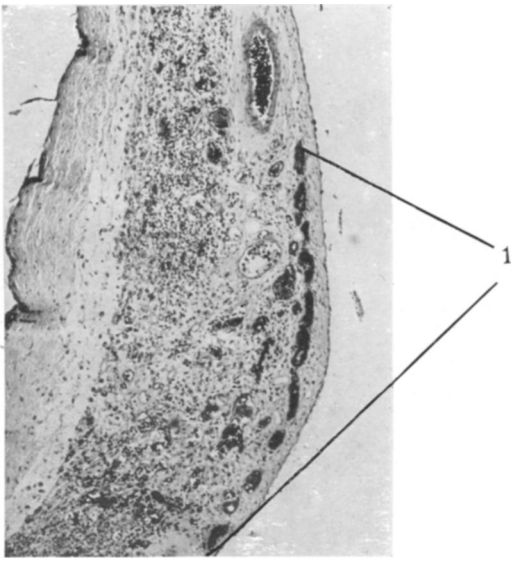


FIG. 9.

PLATE 5.



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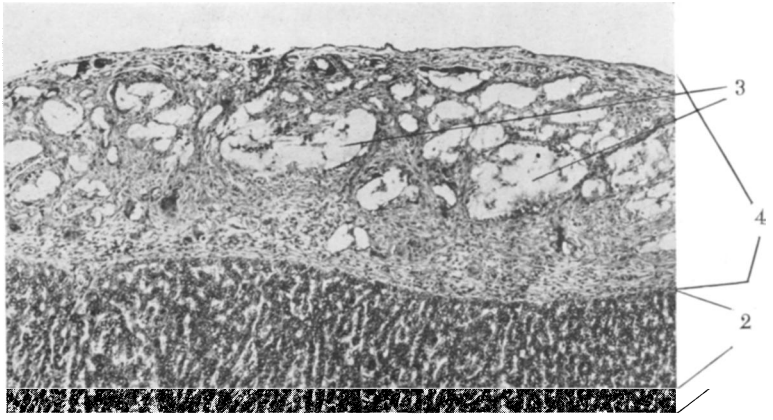
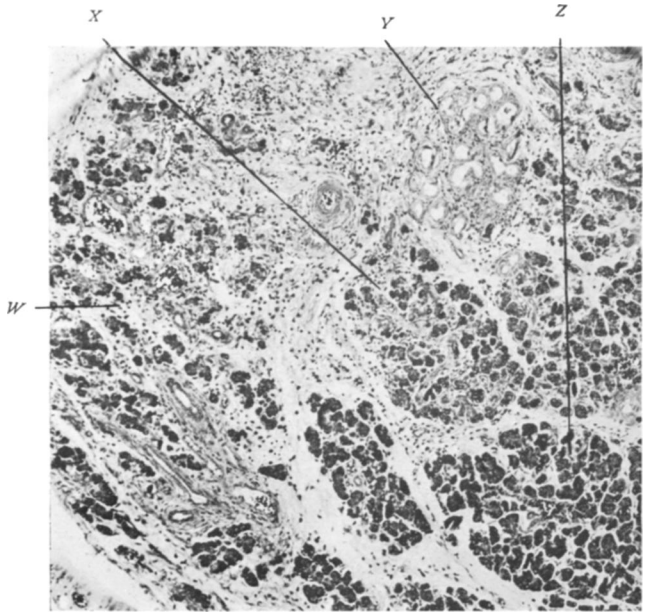


FIG. 11.

PLATE 6.



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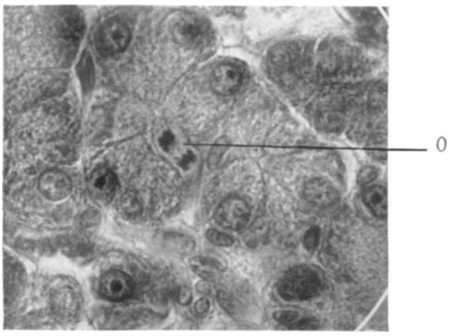

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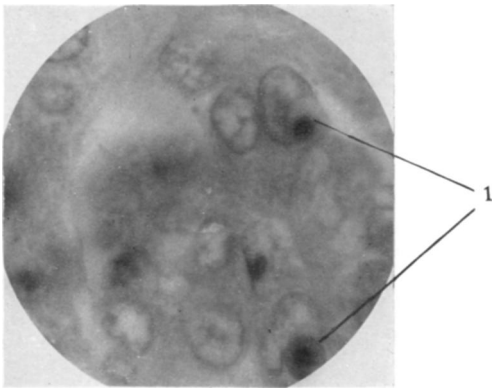

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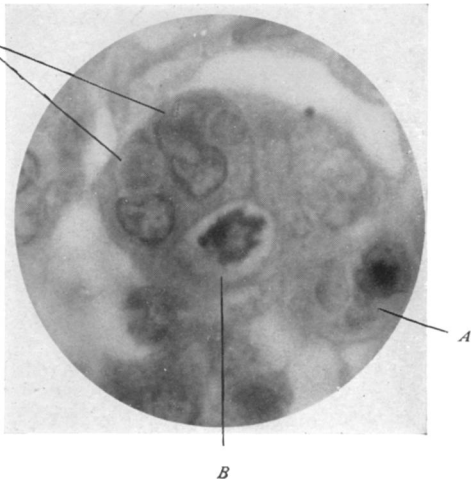

FIG. 15.

PLATE 7.

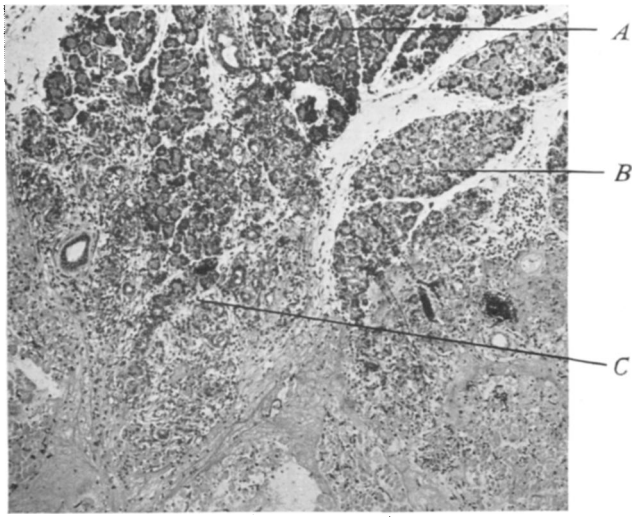

FIG. 16.

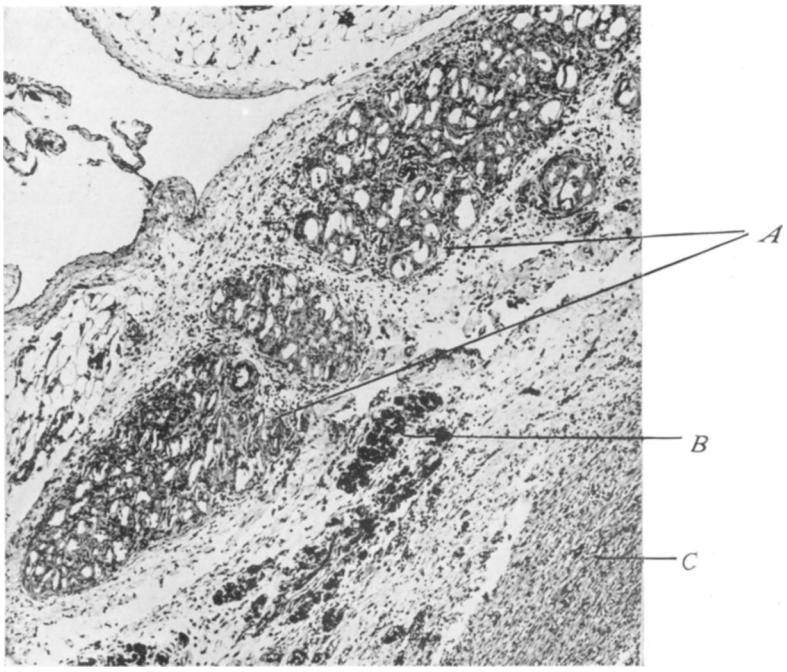

FIG. 17.

PLATE 5.

FIG. 10.—Wall of tissue of capsule surrounding parasitic liver cyst. 1, strand of proliferating liver cells.

FIG. 11.—Peritoneal reaction following injection of plain agar. 2, liver tissue; 3, unorganized agar; 4, peritoneal reaction.

PLATE 6.

FIG. 12.—Reaction following ligature of pancreas and excision (Group B, 3 days). *W*, isolated pancreas acini; *X*, different stages in degenerative metaplasia; *Y*, change of acini to duct type; *Z*, normal pancreas.

FIG. 13.—Mitotic figure in acinus of pancreas (Group B, 10 days).

FIG. 14.—Pancreas cell inclusions (1) from Group B, 10 days.

FIG. 15—Mitotic figure and cell inclusions (*A*) from Group A, 10 days.

PLATE 7.

FIG. 16.—General reaction Group A. *A*, normal pancreas; *B*, degenerative metaplasia; *C*, connective tissue.

FIG. 17.—Pancreas reaction Group A. *A*, duct type of acini; *B*, normal acini; *C*, connective tissue.